Standardization of Epidemiologic Protocols for Surveillance of Acute Diseases Caused by *Streptococcus pyogenes*: Pharyngitis, Impetigo, and Invasive Diseases

Karen Kotloff, M.D.,

Chris Van Beneden, M.D., M.P.H.

Introduction

This document was developed by a working group that was convened following meetings supported by the U.S. National Institutes of Health, and the World Health Organization,to discuss the harmonization of protocols for surveillance of group A streptococcal (GAS) diseases. This protocol addresses three acute manifestations of GAS infection:

- **Pharyngitis**
- **Impetigo**
- **Invasive disease**

For the purpose of this document, GAS refers to pathogenic group A, β-hemolytic streptococci and is used synonymously with *Streptococcus pyogenes*. For each disease, the following issues are discussed and recommendations for harmonization are made:

I. **Case Definition**
II. **Case Ascertainment and Other Surveillance Methodology**
III. **Terms to Describe Disease Burden**
IV. **Core Elements of Case Report Forms**
V. **Specimen Collection**

Two final sections, **Methods of Specimen Transport and Microbiology** and **Quality Management**, contain information that is common to each of the three acute diseases.

Purpose

The purpose of these guidelines is to allow those performing GAS surveillance to standardize data collection to enable comparisons across studies and geographic regions. The definitions and methods presented here may also form the basis for measuring clinical endpoints for future vaccine efficacy trials. In general, these recommendations refer to surveillance for the clinical manifestations of GAS infection among children, although most of the principles may also be applied to surveillance among adults.

Pharyngitis

Background

GAS is the most common bacterial cause of pharyngitis. Although it is difficult to distinguish GAS pharyngitis from nonbacterial pharyngitis on clinical grounds alone, studies of children from temperate climates have shown the following to be suggestive of GAS pharyngitis: age 5 to 15 years, onset during winter season, presence of pharyngeal erythema or exudate, palatal petechiae, tender anterior cervical lymph nodes, fever, and lack of cold symptoms. However, most infections are mildly symptomatic or asymptomatic. In addition, an estimated 20% of children in some studies carry GAS asymptomatically in their throats, sometimes for months at a time. GAS pharyngitis is diagnosed by rapid antigen detection tests or throat culture; the yield of the culture is optimized when performed using standardized methods. Treatment is recommended only for symptomatic infection with the goal of preventing suppurative complications (e.g., peritonsillar abscess) and acute rheumatic fever. In contrast, evidence does not support the ability of antibiotics to prevent acute post-streptococcal glomerulonephritis. This document describes methods that can be applied to achieve the following objectives:

Objectives of surveillance for group A streptococcal (GAS) pharyngitis

Primary objective:

- Determine age-specific incidence or prevalence of symptomatic GAS pharyngitis

Secondary objectives:

- Determine proportion of GAS strains bearing selected genotypic or phenotypic features (i.e., M or *emm* types) (e.g., to measure strain-specific disease burden or potential strain replacement following use of strain-specific vaccine)

- Determine age-specific burden of pharyngitis infections associated with other streptococci, i.e., groups C and G, in order to measure contribution of other β-hemolytic streptococci to overall pharyngitis burden and to measure potential effect of GAS common antigen vaccines on disease due to non-A streptococci

Optional studies that may be incorporated into routine surveillance:

1. Development of algorithms for the use of clinical symptoms to estimate GAS pharyngitis prevalence and, validation of the algorithms with concomitant laboratory confirmation
2. Measurement of antibiotic susceptibility (e.g., to penicillin, macrolides, clindamycin) using MIC (minimum inhibitory concentration) panels or E-test
3. Generation of disease costs, including measurement of primary caretaker time costs and costs of treatment regimens, clinic or hospital stays, and diagnostic tests
4. Passive surveillance for disease sequelae of acute GAS infections

I. Case Definition: Pharyngitis

A case of GAS pharyngitis is defined as a child with the complaint of sore throat from whom GAS (*Streptococcus pyogenes*) is grown in culture who has not met the case definition within the previous 30 days. One must be cognizant of the limitations of this case definition: it does not distinguish colonization from clinical infection. Some experts recommend concomitant measurement of immune responses to assist in making this distinction.

Further discussion of elements of case definition follows:

a. Child

The peak age range for GAS pharyngitis is 5 to 15 years, although cases occur among all age groups. Most protocols are expected to survey school-aged children.

For pharyngitis and other disease outcomes under surveillance, we recommend that findings are expressed as one or more of the age groupings used by international organizations such as the Demographic and Health Surveys of the United States Agency for International Development (DHS/USAID) or the Multiple Indicator Cluster Survey developed by UNICEF. This will both enable comparisons across various protocols and geographic areas and allow for use of local census data from developing countries. The recommended age groups for children are:

- 0-11 months
- 12-59 months (or 12-23 months and 24-59 months)
- 5-9 years
- 10-14 years (or 10-15 years, if matching denominators are available)

b. Complaint of sore throat

To increase case detection and ease of use in countries with few resources, broad clinical criteria should be used. Individual investigators may choose to improve the specificity of the case definition by requiring the presence of clinical features more suggestive of GAS pharyngitis (sudden onset; fever; headache; nausea, vomiting or abdominal pain; inflammation of pharynx and tonsils; patchy discrete exudate; tender, enlarged anterior cervical nodes), the absence of clinical features suggestive of a viral etiology (cough, conjunctivitis, rhinorrhea, diarrhea) and/or by performing anti-streptococcal antibody measurements, but these features make harmonization difficult. All studies should calculate incidence based on the presence of a current complaint of sore throat regardless of other clinical features. To detect current cases of pharyngitis we recommend that investigators ask: "Do you have a sore throat now (today)?" However, it may be appropriate in some settings to ask this question in a different way, and to use other educational aids to make the question easier to understand.

c. Identification of GAS

GAS should be identified via culture, as described below. Although rapid antigen detection tests are commercially available for use on specimens obtained by pharyngeal swab and represent acceptable alternatives to culture in clinical practice, use of these tests do not allow collection of the bacterial strain for further typing. Also, since sensitivity is <100%, it is generally recommended that a culture be performed for GAS if the test is negative and the health care provider suspects GAS pharyngitis. Therefore, culture is the preferred method of GAS identification for surveillance. In addition, some

investigators may seek to determine the frequency of the identification of other β-hemolytic streptococci (e.g., groups C and G) separately using culture.

d. Case definition not met within the previous 30 days

Recrudescent or under-treated cases may return to the investigators in the weeks following an episode of GAS pharyngitis. To avoid counting such episodes as separate cases, new cases must not have been diagnosed with GAS pharyngitis within the previous 30 days. If typing (i.e., M or *emm* typing) capacity is available, a "recurrent" pharyngeal GAS infection may be considered a new case if the M or *emm* typing result indicates a different type than found in the initial case.

II. Case Ascertainment and other Surveillance Methodology

The clinical protocol should clearly describe each of the following elements:

a. Case Ascertainment

The method of case ascertainment is an important part of surveillance and may significantly affect the accuracy and completeness of disease burden estimates. Surveillance is usually active or passive. The definitions of active and passive as used in this protocol are outlined below. The choice to undertake a particular type of surveillance depends on where the study is done, the objectives of the study, and the resources available.

i) "Passive" surveillance, as used in this protocol, is a system of tracking persons with pharyngitis who present to a health care setting. The term "passive" means that the surveillance system relies on the patient initiating contact with the health care or surveillance system. If the provider or investigator determines that the case definition has been met, a report is made to the surveillance system or local public health authorities. Settings where passive surveillance could be performed include outpatient clinics, doctors' offices, and clinical laboratories. Passive surveillance is inexpensive but typically results in significant undercounting of the disease of interest. The use of passive surveillance is best suited for situations when a minimum estimate of disease burden is desired, the population at risk is well-characterized demographically, and most children are taken to a health care facility when they have a sore throat.

ii) "Active" surveillance, as used in this protocol, occurs when public health authorities or study investigators *actively seek* ill persons with the outcome of interest by routinely contacting members of the at-risk population (e.g., through school or household visits. This method is useful in the developing world where many children do not seek care for pharyngitis. Pharyngitis may be regarded as a transient and minor illness and is often untreated or treated only symptomatically. **We strongly recommend that surveillance for pharyngitis be *active* in these settings in order to obtain reliable estimates of the burden of disease.** This would entail contacting a defined cohort of children on a frequent, regular basis for a defined period of time to detect and culture all children with sore throat and is likely to provide the most accurate measure of disease incidence.

b. Participant Eligibility

i) Age: It is expected that children in the peak age range for pharyngitis—age 5 to 15 years—will usually be included, and expressed as described above under *Case Definition*.

ii) Health Status: Unless specifically relevant to the study aims, we *do not* recommend that children with an underlying immunodeficiency or chronic disease be excluded. Also, although

investigators may choose to exclude from surveillance those children receiving prophylactic antibiotics for any cause, we discourage exclusion of children on antibiotics as this history is often inaccurate. Regardless of exclusion criteria, in all studies the protocol should clearly describe the enrollment eligibility criteria.

c. Sampling Frequency

To detect new cases while GAS can still be recovered from a throat culture, we recommend that active surveillance for sore throat be conducted a minimum of once per week. The schedule needed for passive surveillance will vary according to the pattern of case presentation at the study site. The percent of children meeting eligibility criteria and who are enrolled should be recorded.

d. Population at Risk (Denominator from which cases are identified)

The denominator or at-risk population is the defined group of individuals from which cases are identified. The denominator must be well-characterized to derive meaningful estimates of disease burden. For GAS pharyngitis, the population "at-risk" is usually defined by age; the denominator is the number of individuals belonging to each age group under study within the chosen study site.

The denominator also varies with the study design. When performing surveillance in schools, the denominator is the number of at-risk children enrolled in the school. When the entire cohort of children under surveillance attends the participating schools, enrollment records can be used as a type of census. The degree to which the results can be generalized to the entire population of children in the community is affected by the proportion of children who actually attend the participating surveillance site(s) during the defined surveillance period.

Population-based estimates of disease burden may be calculated from some studies. These include household surveillance in a representative sample of children in a community, and surveillance based in a health care setting that serves the entire community. In these cases, the denominator is defined as the total number of eligible at-risk children who reside in the study area. If government-derived census data are used to derive the denominator, the accuracy of the data must be assured. If government census data are not reliable, out-dated, or are unavailable, then the study investigators should perform a census of the eligible at-risk surveyed population. Ongoing demographic surveillance might be necessary if surveillance extends over a long period of time or if the population is not stable because of mobility and other factors. Without an accurate accounting of all children in the sample population who could potentially be evaluated for GAS pharyngitis, incidence may be under- or over-estimated.

e. Time

Defining the period of time during which the sample is surveyed is also crucial for accurate reporting of incidence. It is necessary to record the time *each* child is surveyed for cohort studies in which disease burden will be expressed according to person-time, as described below.

f. Season

For many areas of the world, GAS pharyngitis has seasonal variation. If possible, investigators should perform surveillance across all seasons for at least one full year. Several years of surveillance are necessary in order to elucidate year-to-year variations in incidence and M or *emm* type distribution. Continuous surveillance is optimal, but may not be possible, given the problems of children being required to leave school for either vacation or extended breaks to tend to farms or other family or community duties. In areas where seasonality is already well described, limiting

surveillance to months when most cases are likely to occur may require fewer resources. However, this will not allow calculation of annual incidence unless it has been demonstrated that GAS pharyngitis is not seasonal in the study site.

III. Terms to Describe Disease Burden

The reader is reminded that the case definition does not distinguish between carriers and persons with acute GAS pharyngitis. As a result, some children classified as GAS pharyngitis may actually be asymptomatic carriers experiencing acute pharyngitis due to viruses or other infections.

a. Incidence

Incidence is defined as the number of new cases occurring in a specified period of time per the number of people at risk in the same period. For diseases such as GAS pharyngitis that recur, it is acceptable to count the same child as a case each time he or she meets the case definition during the surveillance period, as long as this is clearly stated. If there is variation of the at-risk group over the specified time period, the denominator is normally set as the mid-point estimate of the at-risk population calculated during that period. Since "at risk" is at least partially defined by age, the denominator should be expressed according to age group, using the categories listed above (see Ia). GAS pharyngitis is a common disease, so incidence can be expressed as cases per 1000 children under study per week or per 100,000 children per year. Enumerating cases per week enables examination of seasonal changes.

b. Cumulative incidence

It is also possible to count incident cases over a specified time period to determine the proportion of individuals in the population who experience a new episode of GAS pharyngitis during that period. In this instance, the numerator is affected children (not episodes), and the denominator is the population at risk at the beginning of the time period. For example, if we survey 80 children in 5th grade for 12 months and detect a new episode of GAS pharyngitis in 8 children, the cumulative incidence of GAS pharyngitis in these 5th graders is 10% for the 12 month period.

c. Incidence Density (Person-Time Incidence Rates)

A person-time incidence determination is useful in cohort studies where individuals are followed for differing but known periods of time. Subjects may both enter or leave the study cohort at different times. To derive the incidence density, the number of incident cases is divided by the total number of person-time units (person-weeks, person-months, etc.) in which all of the individuals in the group were under surveillance. The person-time is the total time the person has been observed. Figure 1 illustrates this principle. Five cases of GAS pharyngitis occurred during 11 + 5 + 6 + 12 + 6 = 40 child-months of observation, yielding an incidence density of 1.5 episodes per 12 child-months.

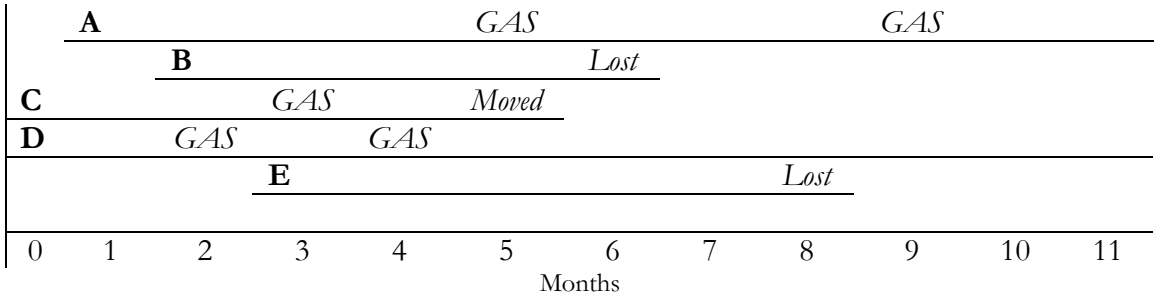

Fig. 1. Schematic representation of a cohort study. Each line from A to E represents the surveillance period for a child.

d. Prevalence

If incidence rates are too difficult or resource-intensive to collect, one may calculate prevalence. Prevalence measures the proportion of the population under study that has the disease of interest at a given point in time; the numerator includes both new and preexisting cases. *Point prevalence* can be calculated for the number of children who meet the case definition of GAS pharyngitis on one particular day. For diseases that have a brief duration, incidence usually approximates prevalence. Exceptions to this rule are infectious diseases such as GAS pharyngitis that have a brief duration but for which infected persons remain susceptible to re-infection. In this case the point prevalence may be low but the annual incidence high. Prevalence estimates for diseases such as GAS pharyngitis that have large seasonal variations can be meaningless, however, unless one samples repeatedly throughout the year. *Period prevalence* may also be calculated; this describes the proportion of the population under surveillance which has pharyngitis during a specified period of time. Because of the brief duration of GAS pharyngitis, period prevalence will be comparable to cumulative incidence.

Note: The term "prevalence of GAS pharyngitis" is sometimes used incorrectly to refer to the proportion or percentage of sore throat cases from which GAS can be cultured. It is recommended that the term prevalence not be used for this purpose.

IV. Core Elements of Case Report Forms for Pharyngitis

Case report forms are used to collect data on enrolled participants. Numerous variables may be included in the case report form, but some core information is essential to perform any epidemiologic study of GAS pharyngitis for the purposes of harmonization across studies and countries. Below are elements that are highly recommended to be included in all case report forms (**bold**) and other suggested elements for possible inclusion (normal type). **When possible, the case report form should include a list of choices rather than an open text field for data capture.**

a. Participant Information

All participants must have a unique identifier to allow for accurate recording of results and to prevent confusion among participants who have repeated encounters. Some methods for facilitating re-identification of participants include photographs, identification cards, and bar code bracelets. All forms used during surveillance must have a place to record the unique identifier on each page.

- **Participant unique ID number**
- **Date** and time **of enrollment**
- **Clinical site** at which child is seen
- Other identifiers such as **name or initials, address**
- **Age (or date of birth) and sex**
- **Episode number** (if repeated episodes from the same person are included)

b. Clinical disease, epidemiology, and treatment

Pharyngitis is clinically defined as acute sore throat (pharyngeal pain). Some children, particularly the older ones, will explicitly complain of throat pain. Others may complain only of pain on swallowing (odynophagia). Study investigators should consider recording the presence or absence of the following signs and symptoms associated with the current episode of pharyngitis. (One expects these symptoms to have begun in the past week):

- **Date of onset of illness**
- *Symptoms*
  - **Throat pain**
  - **Painful swallowing**
  - **Difficulty swallowing**
  - **Fever**
  - **Rhinorrhea (runny nose)**
  - **Cough**
  - Headache
  - Abdominal pain
  - Nausea
  - Vomiting
  - Diarrhea
  - Malaise
  - Chills
  - Hoarseness
- *Signs*
  - **Current temperature _ _ . _ °C**
  - **Erythematous pharynx**
  - **Erythematous tonsils**
  - **Enlarged tonsils**
  - **Tonsillar exudates**
  - **Palatal petechiae**
  - **Enlarged anterior cervical nodes**
  - **Tender anterior cervical nodes**
  - Erythematous uvula
  - Conjunctivitis
  - Coryza
  - Anterior stomatitis
  - Discrete oral ulcers
  - **Scarletiniform rash**
  - Other rash (describe _____)
- *Epidemiologic features of disease, e.g.,*
  - Pharyngitis among family or household members
  - Crowding (number of persons and number of rooms or beds in household)
- *Treatment*
  - Receipt of antibiotics in the past week
  - Antibiotic prescribed at current healthcare visit including dose, frequency, duration

c. Microbiology

- *Specimen collection*
  - **Specimen unique ID** (if more than one specimen is taken, each specimen must have a unique ID number)
  - Date and time specimen obtained
  - **Episode number** (if repeated episodes from the same person are included)
  - **Type (or source) of specimen** (e.g., throat culture, blood culture, RADT)
  - Date and hour plate is inoculated
  - Date and hour plate is placed in incubator
  - Date(s) and hour(s) plate is read
- *Results*

If testing results are not available when the case report form is completed (e.g., if specimens are sent off-site for testing), the results must be matched to the case report form by each participant's unique ID number (plus episode number if repeated episodes from the same person are included).

The simplest database would include:

- **Participant Unique ID number**
- **Date of enrollment**
- **Specimen Unique ID**
- **Group A *Streptococcus* identified: yes/no**

Other possibilities are:

- β-hemolytic *Streptococcus* identified: yes/no
  - *If yes*, group identified (choose one): A, B, C, G or other
- Anti-streptolysin O (ASO) and anti-DNAase B antibodies
- Date of notification of result to participant and/or doctor
- Storage/transport identification number
- Place/site of transfer of isolate for additional testing
- Further testing ordered (e.g., *emm* typing, anti-streptococcal antibody titers, speciation of large-colony β-hemolytic *Streptococcus*, etc.)

V. Specimen Collection: Pharyngitis

a. Equipment and supplies

- Gloves (need not be sterile)
- Sterile swabs (cotton wool or synthetic fiber)
- Tongue depressor
- Flashlight

b. Methods

Proper technique increases the yield of throat cultures in children. Those collecting throat swabs should receive training in the technique which follows:

1. Verify the participant's identity and label a sterile culture swab with the information requested by the protocol. This is typically two patient identifiers, such as initials and study number, the date, and the study or site identity.
2. Put on gloves.
3. Position the child to face the brightest part of the room. If available, have one person steady the child's head.
4. Shine a bright flashlight or penlight in the child's mouth.
5. Remove a swab and hold it with the other hand taking care to keep the tip sterile.
6. Ask the participant to open the mouth widely, protrude the tongue and say, "ahhh" or pant. Swabbing is best done under direct visualization and with the aid of a tongue depressor placed about ¾ of the way to the posterior edge of the tongue to firmly push the tongue down (inferiorly).
7. Rub the swab quickly but thoroughly over both tonsils (or tonsillar fossa) and the posterior wall of the pharynx using light pressure. Any exudate present should be swabbed. Avoid contamination of the swab with saliva, the tongue or oral cavity.

(Please see further guidance below: ***Methods of Specimen Transport and Microbiology***)

Source: http://www.hawaii.gov/health/family-child-health/contagious-disease/influenza/institution_ili/ili_activity_info.htm#3

Impetigo

Background

Impetigo is a highly contagious superficial form of pyoderma. It typically begins as erythematous macules on the skin which rapidly progress to thin-roofed vesicles and pustules, rupture, and form honey-colored crusts. Exposed areas (e.g., face, arms, and legs) are most commonly affected, probably owing to the distribution of the underlying causes of skin disruption (insect bites, minor injury, eczema, scabies, etc.) that allow the organism to penetrate the skin. Impetigo is caused by both group A *Streptococcus* (GAS) and *Staphylococcus aureus*, alone or in combination, with the former predominating in tropical climates. The peak age at diagnosis is 2 to 6 years. Acute post-streptococcal glomerulonephritis is a sequela of GAS impetigo. An association between impetigo and rheumatic fever has not been established, although some investigators have suggested a link. Effective treatment of children with GAS impetigo must address the underlying scabies or other skin lesions. Although most impetigo lesions will resolve spontaneously within several weeks, specific systemic or topical antibiotics speed recovery but do not appear to prevent nonsuppurative sequelae. This document describes methods that can be applied to achieve the following objectives:

Objectives of surveillance for GAS impetigo

Primary objective:

- Determine age-specific incidence or prevalence of GAS impetigo

Secondary objectives:

- Describe the genotypic or phenotypic (i.e., M or *emm* types) distribution of GAS strains causing GAS impetigo (e.g., to measure strain-specific burden or potential strain replacement following introduction of strain-specific vaccines)

I. Case Definition: Impetigo

A prevalent case of GAS impetigo is defined as a child who has one or more vesiculopustular or crusted ulcer eruptions from which GAS (*Streptococcus pyogenes*) is grown in culture. An incident case is the presence of one or more culture-positive lesions in a child who did *not* have any lesions at the previous visit. We recommend that an episode is considered ongoing as long as vesiculopustular or crusted ulcer lesions are present; in this situation additional culture confirmation is not necessary. A case is considered resolved when the child has no lesions. In areas of high impetigo incidence, a child may have new lesions appearing frequently while others resolve. Using the above definition of incident cases, this child will have lesions present at two consecutive visits and will therefore not be considered to be an incident case at the second visit even though new lesions have appeared in the interim and the original lesions may have resolved. It is very difficult to account for this phenomenon in epidemiologic studies of incidence except by detailed review (such as photography) of each lesion, which is not practical. Therefore, the above definition of incident cases will be

strengthened if researchers treat children with impetigo with appropriate antibiotics followed by re-examination to ensure that lesions have resolved.

Further discussion of elements of the case definition:

a. Child

The peak age range for GAS impetigo is 2 to 6 years, but cases occur at all ages. Most protocols are expected to survey children in this age range. The age groups should be predefined in the protocol and expressed in the categories outlined above in *Case Definition: Pharyngitis*.

b. Vesiculopustular or crusted ulcer eruption

To increase the sensitivity of case detection, the clinical criteria for obtaining a culture should be broad, encouraging culture of as many cases of impetigo as possible. Investigators could improve the specificity of the case definition by requiring presence of other clinical features that are most characteristic of GAS impetigo (e.g., absence of systemic signs or symptoms; presence of honey crusts), but these features are difficult to harmonize. Therefore, all studies should seek to identify clinical cases of impetigo based on the presence of any vesiculopustular or crusted ulcer eruption for which an alternate diagnosis has not been determined.

c. GAS is grown in culture

Only impetigo infections from which GAS are identified by culture are to be considered cases. Cultures are also necessary in order to further characterize the GAS strain. **The use of rapid diagnostic tests for GAS impetigo in surveillance studies or as part of the harmonized case definition is discouraged.**

II. Case Ascertainment and other Impetigo Surveillance Methodology

The clinical protocol should clearly describe each of the following elements:

a. Case Ascertainment

As with pharyngitis, surveillance can be active or passive (see discussion of *Case Ascertainment* in the *Pharyngitis* section above). Passive surveillance is likely to significantly underestimate the burden of impetigo due to GAS as most children due not seek care for impetigo. The most desirable method to ensure case detection is active surveillance of a defined cohort of children or families with close follow-up for a defined period of time, recognizing the disadvantages posed by expense and retention difficulty over prolonged periods. Given that the peak age of GAS impetigo occurs before school entry, the use of elementary schools as the sole sites of surveillance will underestimate the true disease burden and should be complemented by settings where younger children can be captured (e.g., household surveys).

b. Participant Eligibility

i) Age: Age eligibility may vary from site to site, but children in the peak disease age range of 2 to 6 years should be included. Age should be expressed in the groups described above under *Case Definition: Pharyngitis*.

ii) Health Status: Unless otherwise relevant to the study aims, we *do not* recommend exclusion of children with an underlying immunodeficiency or chronic disease. Also, although investigators may choose to exclude those children already receiving prophylactic antibiotics for any

cause, we discourage exclusion of children on antibiotics as this history is often inaccurate or poorly remembered. In all studies, the protocol should clearly describe the enrollment eligibility criteria.

c. Sampling Frequency

Children under active surveillance should be visited at least once a week to detect new cases and determine the duration of lesions. The schedule of passive surveillance will be dictated by the pattern of case presentation at the study site.

d. Population at Risk (Denominator from which cases are identified)

The denominator is the total number of eligible at-risk children from which cases are identified. The denominator must be well-characterized to derive meaningful estimates of disease burden. For GAS impetigo, the most commonly identified at-risk population will be preschool and early school-age children. **If the aim of the study is to measure the incidence of new cases, children with lesions at the start of the study must be excluded from the denominator.** These children can be counted (e.g., in a person-time analysis) once they become free of lesions.

When performing surveillance in classrooms, the denominator is the number of at-risk children enrolled in the school. When the entire cohort of children under surveillance attend the participating schools, the enrollment records can be used as a census, and the results can be generalized to the community. Otherwise, the degree to which the results can be generalized to the entire community is affected by the proportion of children who actually attend the participating surveillance site(s) during the entire defined surveillance period.

Some other types of studies allow population-based estimates of disease burden to be calculated. These studies include household surveillance in a representative sample of children, or surveillance based in a health care setting that serves the entire community. The denominator is defined as the total number of eligible at-risk children who reside in the study area. If government-derived census data are used to derive the denominator, the accuracy of the data must be assured. If government census data are not reliable, out-dated, or are unavailable, then the study investigators should perform a census of the eligible at-risk surveyed population. Ongoing demographic surveillance might be necessary if surveillance extends over a long period of time or if the population is not stable because of mobility and other factors. Without an accurate accounting of all children in the sample population who could potentially be evaluated for GAS impetigo, incidence may be under- or over-estimated.

e. Time

A defined time period during which the sample is surveyed is also crucial for accurate reporting of incidence. It is necessary to record the time *each* child is surveyed for cohort studies in which disease burden will be expressed according to person-time, as described below.

f. Season

For many areas of the world, GAS impetigo has seasonal variation, often with peaks of incidence in the rainy, hot months. To capture seasonal changes, investigators should perform surveillance across the seasons, if possible. If attempting to calculate incidence, continuous surveillance for at least 12 months is optimal. Several years of surveillance are necessary if one wishes to elucidate year-to-year variations in incidence and M or *emm* type distribution. Continuous surveillance is optimal, but may not be possible, given the problems of children being required to leave school for vacation and sometimes for extended breaks to tend to farms or other family or community duties.

In areas where seasonality is already well described, limiting surveillance to months when most cases are likely to occur has efficiencies, and may provide reasonable estimates of the true incidence.

III. Deriving Terms to Describe Disease Burden

a. Incidence

Incidence is defined as the number of new cases occurring in a specified time period per the number of people at risk in the same period. GAS impetigo can be an acute, self-limited disease or an indolent, recurrent, or chronic affliction with multiple lesions at various stages of resolution over months or years. Determining the incidence of impetigo is challenging because of the difficulty in distinguishing a new episode from a lingering previous episode. If children are surveyed frequently, then it should be possible to document that they are free of lesions at a given visit, after which the appearance of a characteristic lesions that grow GAS would constitute a new episode.

For diseases such as GAS impetigo that recur, it is acceptable to count the same child as a case each time he or she meets the case definition during the surveillance period, as long as this is clearly stated. If there is variation of the at-risk group over the specified time period, the denominator is normally set as the mid-point estimate of the at-risk population calculated during that period. The denominator should be expressed according to age group. Where GAS impetigo is a common disease, incidence can be expressed cases per 100 children under study. Enumerating cases per week enables examination of seasonal changes. However, incidence rates may also be described in other units of person and time (e.g., new cases per 1000 children per month).

b. Cumulative incidence

It is also possible to count new (incident) cases over a specified time period to determine the proportion of individuals in the population who become affected during that period. In this instance, the numerator is the number of children who have a new episode while participating in the surveillance study, and the denominator is the population at risk and free of impetigo at the beginning of the time period. For example, if we survey 80 children in 1st grade for 12 months and detect a new episode of GAS impetigo in 60 children, the cumulative incidence of GAS impetigo in 1st graders is 75% for the 12 month period. In this particular analysis, children with recurrent episodes would only be counted once.

c. Incidence Density (Person-Time Incidence Rates)

A person-time incidence determination is useful in cohort studies where individuals are followed for differing but known periods of time. The details of how this outcome is computed are described above in the section on *Pharyngitis* and in Figure 1.

d. Prevalence

There are advantages to using prevalence rather than incidence to measure disease burden from GAS impetigo in tropical environments. *Point prevalence* measures the number of children who have impetigo at one specific instance in time (i.e., a particular day). Both existing cases and new cases are counted in the numerator, and all at-risk children are counted in the denominator regardless of whether they have existing lesions. This measure does not require the investigators to define the beginning and end of an episode and offers economies of time and expense. However, because GAS impetigo may have large seasonal variations, point prevalence measurements should be repeated periodically throughout the year.

Another measure of disease prevalence is the total number of days of observation during which the children in the cohort experience impetigo, adjusted for different periods of follow-up if necessary (e.g., percentage of observation days with impetigo). This outcome could be expressed categorically using reasonable time intervals (e.g., the number of children who experienced impetigo on the following percentages of observation days: 0, 1-5%, 6-10%, 11-25%, 26-50%, 51-75%, >75%) or as a mean or median of the percent of affected days experienced by each child.

e. Course of an Episode: Duration and Severity

For investigations concerning the course of the episode after the onset of illness, the outcome measures may be quantitative (duration) or qualitative (severity of the episode) depending on the study question. Only children who suffer an episode of impetigo contribute information to this analysis. For example, for each child one could calculate the mean duration per episode of impetigo and express the results categorically using reasonable time intervals. The results could also be expressed as a mean or median.

Some investigators have used the following scale to grade the severity of impetigo from 1 to 18. [1] The results of such an analysis could be expressed in categories or as the mean severity score for each child. Severity scales require validation before their use can be recommended as a primary endpoint in an intervention trial.

Score	1	2	3	Maximum score/person/category
Type of sores	Flat/dry	Crusted	Purulent	6
No. of lesions				
Upper limbs	<5	5-20	>20	3
Legs/buttocks	<5	5-20	>20	3
Trunk	<5	5-20	>20	3
Head/neck	<5	5-20	>20	3
Max. total score per person				**18**

IV. Core Elements of Case Report Forms for Impetigo

Below are elements that are highly recommended to be included in all case report forms (bold) and other suggested elements for possible inclusion (normal type). It is always best, when possible, to provide a list of choices rather than an open text field for data capture.

a. Participant Information

- **Participant unique ID number**
- **Date and time of enrollment**
- **Site** at which child is seen (e.g., clinic, school, household)
- Other **identifiers** such as **name or initials, address**
- **Age (**or **date of birth)** and **sex**
- **Episode number** (if repeated episodes from the same person are included)

b. Clinical disease, epidemiology and treatment

Signs and symptoms of particular clinical disease in past week: choice fields are best. For impetigo, the following should be considered:

- **Date of onset of lesions**
- *Symptoms:*
  - **Fever**
    - Concurrent sore throat
- *Signs:*
  - Current temperature _ _ . _ °C
  - **Number of lesions**
    - Upper extremities
    - Lower extremities
    - Trunk/back
    - Head/neck
  - Type(s) of lesion(s)
    - Bullous
    - Wet/purulent
    - Crusted/scabbed
    - Flat/dry (healing)
- *Epidemiologic features, e.g.:*
  - **Underlying skin disease (e.g., scabies, eczema, insect bites, trauma, other)**
  - Impetigo among family or household members
  - Crowding (i.e., number household members, number of bedrooms in household)
  - Receipt of antibiotics in the past week
- ***Treatment***
  - Antibiotic (**topical, oral, parenteral**) prescribed (dose, frequency, duration)

c. Microbiology

- *Specimen collection*
  - Date and hour plate is inoculated
  - Date and hour plate is placed in incubator
  - Date(s) and hour(s) plate is read
- *Results*
  - **Group A *Streptococcus* identified: yes/no**

Other possibilities are:

- β-hemolytic *Streptococcus* identified: yes/no
  - If yes, choose one of the following groups: A, B, C, G, other
- Other organism identified: yes/no
  - If yes, choose one: *Staphylococcus aureus*, other
- GAS identified from throat culture (serial, simultaneous collection of skin and throat specimens for culture may be performed to elucidate whether skin strains proceed to infect the throat or vice versa)
- Date of notification of result to participant and/or doctor
- Storage/transport identification number

- Place/site of transfer of isolate for further testing
- Further testing ordered, e.g., *emm* typing, confirmation, further identification of large-colony β-hemolytic *Streptococcus* to the species or subspecies level, etc.

V. Specimen Collection: Impetigo

a. Equipment and supplies

- Gloves (need not be sterile)
- Sterile swabs (cotton wool or synthetic fiber)
- 70% alcohol
- Sterile needles

b. Methods

1. Use a sterile swab (packaging must be intact).
2. Verify the identity of the participating child and label a sterile culture swab with the information requested by the protocol (typically two patient identifiers such as initials and study number), the date, and an identifier for the person plating the organism).
3. Put on gloves.
4. If there are multiple lesions, sample up to 3 lesions, choosing those that are fresh; older lesions are more likely to become co-infected with *Staphylococcus aureus* or to fail to grow any organisms.
5. The method of sample collection depends upon the character of the impetiginous lesion:
   a. *For dry non-vesicular lesions without a crust*, the swab itself should be moistened with sterile water or saline and then rubbed with moderate vigor over the lesion.
   b. *For dry non-vesicular lesions with a crust*, one of 2 techniques may be used:
      i. Wipe the crust with 70% alcohol, sterile saline or sterile water and then carefully remove the crust with either a sterile instrument, such as a scalpel or needle, or another suitably sharp, clean object. If using swabs with a long, wooden shaft, the shaft can be broken and the sharp end of the broken shaft used for this purpose. With moderate vigor, rub the base/floor of the uncrusted lesion with a swab moistened with sterile water or saline
      ii. Use your fingers to stretch the skin at both edges of the crust to express a drop of purulent material from underneath, which is collected on the tip of a dry swab. The swab should not touch the crust or skin. This technique is considered by some less sensitive than rubbing of the base of the lesion, since pus typically contains fewer viable organisms.
   c. *For dry vesicular lesions,* wipe the vesicle with 70% alcohol, sterile saline or sterile water. Puncture the vesicle aseptically with a needle and swab the serous or purulent fluid.
   d. *For wet lesions*, directly swab the lesion.
6. Use swabs from packages with intact wrapping and take care to keep the swab tip sterile.
7. Avoid touching normal skin with the swab.

(Please see further guidance below: ***Methods of Specimen Transport and Microbiology***)

Invasive Group A Streptococcal Diseases

Background

Invasive group A streptococcal (GAS) infections occur when the organism invades a normally sterile site in the body, producing conditions such as bacteremia, necrotizing fasciitis, lymphangitis, myositis, osteomyelitis, and septic arthritis. Case fatality of invasive GAS disease is high, particularly when accompanied by necrotizing fasciitis or streptococcal toxic shock syndrome (STSS), a syndrome defined as hypotension in combination with at least two of the following: acute renal failure, coagulation or liver abnormalities, rash, respiratory distress syndrome, and soft tissue necrosis. Incidence peaks in the very young and the very old. Even with aggressive antimicrobial and surgical treatment, mortality of invasive GAS infection remains high, particularly in adults and when there is concomitant STSS.

Objectives of surveillance for invasive GAS infections

Primary objective:

- Determine age-specific incidence of invasive GAS infections

Secondary objective:

- Describe the genotypic or phenotypic (i.e., M or *emm* types) distribution of GAS strains causing invasive GAS disease (e.g., to measure strain-specific burden or measure potential strain replacement following introduction of strain-specific vaccines

I. Case Definition: Invasive GAS Infections

A case of invasive GAS disease is defined as isolation of GAS from a normally sterile site or from a wound culture or tissue specimen in a patient who develops necrotizing fasciitis or STSS. Criteria for diagnosis of both confirmed and probable STSS have been defined by The Working Group on Severe Streptococcal Infections.[2] A normally sterile site contains no microorganisms in a healthy state and includes, but is not limited to, blood, cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid, pericardial fluid, bone, muscle, joint fluid, and internal organs (e.g., lymph node, brain, heart, liver, spleen). For the purpose of this surveillance we recommend the inclusion of women who develop clinical signs of postpartum endometritis and for whom GAS is isolated from the cervix.

Cases should be identified by culture. Some surveillance systems also include the presence of Gram positive cocci, antigen-detection or other non-culture based methods, or serologic confirmation of GAS infection in addition to culture positivity in the case definition for invasive GAS infection. However, phenotypic or genotypic characterization of the causative GAS strain is not possible if cases are detected by these methods.

It is important to record each case of invasive GAS disease, but not to record any case more than once. Confusion could arise, for example, when a patient has an illness during which GAS grows from multiple body sites, such as blood and pericardial fluid. Even though that patient has more than one diagnosis (pericarditis and bacteremia), his/her illness should be counted as a case of invasive GAS disease only once.

Because GAS may colonize the skin, throat and vagina without causing illness, isolation of GAS solely from one of the following sites would **not** meet the case definition for the purpose of this surveillance: middle ear, amniotic fluid, placenta, throat, sinus, lung, gallbladder, appendix, cornea, wound, skin abscess, or subcutaneous tissue—unless the patient also has necrotizing fasciitis or STSS.

II. Case Ascertainment and Other Surveillance Methodology

a. Case Ascertainment

Invasive GAS disease is typically an acute, often life-threatening disease. In contrast to pharyngitis and impetigo, the majority of patients with invasive streptococcal disease will seek medical care. Case finding for invasive GAS infection may therefore be either passive or active. Passive surveillance may be adequate if routine medical care of patients with potential invasive GAS infection or any febrile illness includes blood culture or culture of the appropriate normal sterile site (e.g., joint aspiration and culture of a septic joint) and if the subsequent culture technique is adequate. In this case providers can simply be instructed to report all invasive GAS infections to the study investigator.

Surveillance for invasive GAS infections is typically achieved through routine, thorough, periodic review of hospital laboratory records for identification of positive GAS cultures from normally sterile body fluids. Where possible, it should be further augmented through review of medical records or death certificates for clinical syndromes typical of invasive GAS (e.g., necrotizing fasciitis, STSS).

Complete case ascertainment may be improved by instructing the hospital staff to ensure that the appropriate cultures are obtained on patients with the following syndromes:

- Necrotizing fasciitis (wound and blood cultures)
- STSS (blood, throat and wound cultures, if appropriate)
- Puerperal sepsis (blood, speculum cultures)
- Arthritis or septic joint (blood and joint cultures)
- Meningitis (cerebrospinal fluid)
- Cellulitis, erysipelas, lymphangitis, osteomyelitis, etc. (blood culture, sterilely obtained tissue aspirate and culture)
- Pneumonia (blood, thoracentesis with sterilely obtained culture of pleural fluid)

Since isolation of GAS from a normally sterile site (or from wounds if in a patient with STSS or necrotizing fasciitis) is essential to the case definition of invasive disease, the microbiology laboratories in acute care hospitals and reference laboratories processing sterile site specimens for residents of the surveillance area are the most efficient sites for case identification.

b. Participant Eligibility

(i) Age: Age eligibility may vary from site to site, depending on local needs, but it is expected that all children will usually be included. Many sites will include all ages as adults often have more severe outcomes of invasive GAS infections. Regardless of the age groups chosen, they should be expressed as age groupings used by DHS/USAID supported surveys. This will enable comparisons across protocols and geographic areas and allow use of local census data from developing countries. Please see recommended age groupings in *Case Definition: Pharyngitis* above.

(ii) Health Status: The underlying health status of children or adults under surveillance may or may not be recorded. Ideally, data about the past medical history of the patient will be available and recorded on the case report form. Persons with underlying immunocompromising or chronic diseases should not be excluded from surveillance.

c. Sampling Frequency

Case ascertainment can be improved if the investigators make regular visits to the study sites to re-educate clinicians about obtaining appropriate cultures for patients who meet the clinical case definitions and to review clinical and laboratory records to detect missed cases.

d. Active follow-up

The extent of follow-up will be determined by the specific protocol. Data collected about invasive GAS infections should include hospital mortality and a range of severe morbidities (e.g., renal failure, amputation), when possible.

e. Population at risk (Denominator from which cases are identified)

The denominator or at-risk population will be all residents of the hospital or laboratory catchment area. The surveillance will typically occur in a defined geographic or medical clinic catchment area served by the laboratory performing the cultures, and therefore the denominator must be defined as the total number of eligible at-risk people. If government-derived or health care facility calculated census data are used, the accuracy of the data must be assured. If census data are not reliable or are unavailable, then the study investigators should perform a census of the eligible at-risk surveyed population themselves. Without an accurate accounting of all people in the sample population who could potentially be evaluated for invasive GAS disease, incidence may be under- or over-estimated. If cases do not reside in the defined catchment area, they should be excluded. Ideally, the denominator population should be defined before surveillance begins.

f. Time

A defined time period during which the sample population is surveyed is also crucial for accurate reporting of incidence. The amount of time used to follow a cohort of people will vary depending on the sample size, needs and resources of the study. For invasive GAS disease, it is recommended that incidence be expressed as cases per 100,000 population per year.

g. Season

Although invasive GAS disease exhibits seasonal peaks and troughs in North America and Europe, this is not the case in all countries. Investigators should consider performance of surveillance across all seasons, if possible. Given the low incidence of invasive disease relative to GAS pharyngitis and impetigo, longer periods of surveillance are generally required to determine accurate estimates of incidence and of organism type distribution. Continuous surveillance for at least 12 months is optimal. Several years of surveillance are necessary if one wishes to elucidate year-to-year variations in incidence and M or *emm* type distribution.

III. Deriving Terms to Describe Disease Burden

a. Incidence

Incidence is defined as the number of new cases occurring in a specified period of time per the number of people at risk in the same period. To determine incidence of disease, the "numerator" (number of cases), the "denominator" (at-risk, surveyed population sample), and the time under surveillance must be accurately recorded.

In population-based surveillance each case must satisfy the case definition AND come from the denominator population to obtain accurate calculations of incidence. This can be a problem where surveillance is conducted in a hospital that provides tertiary level care as these hospitals may admit patients with invasive GAS infections from other regions. Therefore, it is critical to determine if the

residential address of people with invasive GAS infections is also the same as the place they have been residing over the previous 30 days.

c. Cumulative incidence

It is also possible to count incident cases over a specified time period to determine the proportion of individuals in the population who experience an episode of invasive GAS disease during that period. In this instance, the numerator is affected individuals, and the denominator is the population at risk at the beginning of the time period. For example, if we conduct passive surveillance for 12 months in a hospital serving a population of 500,000 and detect a new episode of invasive GAS disease in 50 people, the cumulative incidence of invasive GAS disease in this population is for the 12 month period is 1 per 10,000 population. In contrast to other GAS diseases, invasive disease tends not to be amenable to expression as incidence density or as prevalence.

IV. Core Elements of Case Report Forms

Case report forms are used to collect data on enrolled participants. Numerous factors may determine the actual content of the case report form, but some information may be considered required to perform any epidemiologic study of invasive GAS disease for the purposes of this harmonization. Below are elements that are highly recommended to be included in all case report forms (bold) and other suggested elements for possible inclusion (normal type).

All participants must have a unique identifier to allow for accurate recording of results and to prevent confusion among participants who have repeated encounters. Some methods employed for this purpose include photographs, identification cards, and bar code bracelets. All forms used during surveillance must have a place to record the unique identifier, ideally on each page.

a. Participant Information

- **Participant unique ID number**
- **Date** and **time of enrollment/culture**
- **Clinical site** at which patient is seen
- Other **identifiers** such as name or initials, address
- **Age (**or **date of birth), sex, race/ethinicity**
- Occupation
- Recent travel

b. Clinical disease

- **Type of infection** (check all that apply):

▫ streptococal toxic shock syndrome	▫ abscess	▫ chorioamnionitis
▫ sepsis	▫ cellulitis	▫ puerperal sepsis
▫ bacteremia	▫ osteomyelitis	▫ endometritis
▫ meningitis	▫ septic arthritis	▫ septic abortion
▫ endocarditis	▫ necrotizing fasciitis	▫ peritonitis
▫ pericarditis	▫ pneumonia	▫ empyema

▫ other (specify _____)

- **Risk factors for invasive GAS disease**

- **Underlying illnesses or other risk factors for invasive GAS disease** (e.g., chronic heart or lung disease, HIV infection or AIDS, cancer, diabetes, chronic steroid use, high doses of short term steroid treatment, intravenous drug use, recent surgery, recent delivery of baby, varicella infection, penetrating trauma
- Other pertinent epidemiologic features of disease such as:
  Crowding (number of persons and rooms or bedrooms per household)
  Pharyngitis among family or household members

- **Treatment**
  - Surgery: amputation or debridement, (specify)
  - Antibiotic prescribed, dose, frequency, duration
  - Use of intravenous immune globulins
  - Clinical outcome (e.g., renal failure, intubation, placement in intensive care unit, amputation of limb, death)

c. Microbiology

- **Specimen collection**
  - **Unique specimen ID**
  - Date and hour culture obtained
- **Results**
  - Date and time identification of GAS confirmed
  - Other organisms identified
  - Site from which culture was obtained (with choice list including but not limited to: blood, wound, joint, CSF, peritoneal fluid, etc.)
  - Date of notification of result to participant and/or doctor
  - Storage/transport identification number
  - Place/site of transfer to another facility for further testing
  - Further testing ordered, e.g., *emm* typing, confirmation, antimicrobial susceptibility, etc. (if needed)

V. Specimen Collection: Invasive GAS Infections

a. Equipment and supplies

- Sterile gloves
- **Sterile equipment** for obtaining blood culture, lumbar puncture, etc.

b. Methods

Collection of specimens from normally sterile sites should be performed in a standardized fashion by all investigators. These specimens might include swabs (e.g., from wounds or fasciitis sites), blood from venipuncture, aspirated body fluid or purulent material, or tissue (e.g., biopsy material from fasciitis site). Personnel should be trained in the performance of the sterile procedures required (i.e., blood culture, lumbar puncture, joint fluid aspiration, etc.) and a standard operating procedure for obtaining, processing, transporting, and storing isolates should be written, approved, and followed. Proper technique will increase the yield of normally sterile site cultures. If such training and practice is not feasible, investigators will rely on local clinical practices.

Methods of Specimen Transport and Microbiology

The methods described in this section are adapted from *Laboratory Diagnosis of Group A Streptococcal Infections, WHO 1996*. The intention is to provide an overview. The reader is referred to this book for media preparation recipes and more detailed descriptions of the methods for cultivating GAS.

a. Specimen transport - swabs

Immediate inoculation of specimens onto blood agar plates is optimal, but requires the ability to maintain a supply of quality-controlled plates in refrigerator storage at the site where patients are seen and to ensure safe transport of inoculated plates if the laboratory facility is located elsewhere. If the time between specimen collection and plating is greater than a few hours, a transport medium (e.g., Stuart's medium), the filter-paper strip method or the silica-gel transport system is recommended. If plating will be delayed more than 48 hours, then the filter paper strip method or the silica-gel transport system should be used. It is important to note that high temperature and high humidity can adversely affect viability of GAS if plating is delayed. Therefore, special precautions must be taken under these conditions (see below).

i. *Transport media:* Culture transport systems containing Stuart's medium are commercially available. They are usually composed of a plastic tube with media in the tip. The media is released once the swab is returned to the plastic sleeve, often by crushing the tip. Stuart's medium can also be prepared in the laboratory. Although high humidity should not affect viability of GAS in transport media, the effects of elevated temperature are unknown. Therefore, it is prudent to transport such specimens to the laboratory in a "cold-box" (e.g., a Styrofoam box containing a frozen ice pack).

ii. *Filter paper strip method:* Filter paper strips transport kits are simple to prepare. The specimen swab is rolled along the filter paper, dried for 5-15 minutes, and sealed in aluminum foil. Plating can be postponed for up to 7 days as long as conditions are not humid. In humid conditions, the filter paper samples should be kept and shipped in a closed container with a desiccant (silica gel). Under conditions of "normal" temperature and humidity, the kit can be mailed in an ordinary envelope if postal regulations permit. Note: Filter paper strips should be handled with sterile forceps.

iii, *Silica gel desiccated swabs*: Placing the swab directly into a transport tube containing silica get desiccant is useful in humid environments when immediate plating is not possible. Silica gel desiccated swabs are not commercially available; silica gel transport system can be prepared by placing an amount of silica gel crystals in a test tube that is enough to cover the head of the swab (about 15 mm deep). If the silica gel crystals are handled carefully to avoid contamination the tube and crystals do not need to be sterilized. After inserting the swab specimen into the silica gel, the tube must be tightly sealed for transport. Silica gel transported swabs may be directly plated when received in the laboratory or quickly dipped in a tube of broth medium (Todd-Hewitt broth) and then plated. The swab may then be placed in the broth medium for a minimum of four hours (overnight incubation is satisfactory) for follow-up culturing onto a blood agar plate (see Enrichment Broth below).

iv. *Transport on agar:* Immediately plating the swab onto blood agar plates can yield excellent results under ideal conditions. However the plates must be packaged in a way that offers protection from damage due to rough handling and from temperature extremes (either

elevated or freezing temperatures). Transport of specimens on agar should be limited to situations where arrival and processing in the laboratory occurs either the same day (preferable) or within 24 hours.

v. *Desiccant method of transport*: Inoculated swabs may be returned to their original packaging (e.g., plastic tube or a paper sleeve) and sealed to protect from contamination. After labeling, up to 20 swabs can be placed in a sealed plastic bag with one sachet of commercially–available silica desiccant. For transport, one or more of these plastic bags can then be placed with a frozen freezer-block into a standard cooler box (e.g. made of polystyrene foam). The freezer block must not directly contact the swabs; this may be avoided by wrapping in bubble wrap. The recovery of GAS from swabs using the desiccant method has been validated in tropical settings and is nearly as effective as direct plating. [3]

b. Specimen transport: fluids and tissue

Aspiration into a syringe is the standard method of sampling body fluid collected from a normally sterile site, abscess fluid, or other localized GAS infection. Biopsy material may also be obtained, e.g., in the diagnosis or treatment of necrotizing fasciitis. These fluid and tissue specimens should be immediately transported to the laboratory in a sterile container (but never with a needle attached). Tissue specimens must be kept moist by adding a few drops of sterile, nonbacteriostatic saline to the container or by placing the sample on a sterile piece of moistened gauze. Fluid or pus may also be sent on swabs and transported to a microbiology lab as described in **a.** above.

c. Culture media

i) *Sheep blood agar:* Tryptic soy agar with 5% sheep blood, commonly known as sheep's blood agar (SBA), is commonly used to grow GAS. Use of horse and rabbit blood is also satisfactory but agar containing human blood is generally not acceptable. Bloods from some other mammalian species have been used successfully, but, if local circumstances dictate use of such alternatives, it is imperative that the blood be thoroughly and carefully evaluated as described below. If blood is obtained locally, one must be sure that the animals are not fed antibiotics. Whether prepared in the lab or purchased from a vendor, each new batch should be tested for its ability to support good growth of β-hemolytic streptococci with appropriate hemolytic and morphological characteristics. Plates should be stored at 4°C in sealed plastic bags to prevent drying and warmed to room temperature just before use. Expired plates or plates showing any signs of darkening or hemolysis should not be used. Users of horse or rabbit blood should be aware that beta-hemolytic *Haemophilus* species grow on these blood agar plates and colonies of such may resemble beta-hemolytic streptococci.

ii) *Selective agar media:* Commercially made blood agars selective for streptococci are available but generally more expensive than nonselective media. They typically contain crystal violet or the antibiotics trimethoprim (TMP)/sulfamethoxazole (SMX) (1.25 mcg/mL TMP and 23.75 mcg/mL SMX) or colistin and nalidixic acid. These media may be used if the user is experiencing heavy contamination of swabs. If antibiotic impregnated agars are used, researchers should be aware that growth of GAS will be slower and colonies may be smaller and with smaller zones of hemolysis.

iii) *Selective or enrichment broth:* For detection of bacteria in samples likely to contain low colony counts, such as pus, recovery of GAS can be enhanced by supplementing primary plating with either selective broth (containing antibiotics or other agents to inhibit growth of non-streptococcal species) or enrichment broth (serum broth, or blood broth). Enrichment broths can be any bacteriological growth medium yielding good growth of GAS (e.g., Todd Hewitt, trypticase

soy, brain heart infusion) with or without addition of 5% defibrinated animal blood or serum. The swab is placed in the broth, incubated overnight at 35-37°C, and then inoculated using a wire loop or 10 µl plastic loop onto a fresh SBA plate.

d. Inoculation of plates (see Figure)

It is preferable to use an entire SBA plate for each swab specimen. First inspect the plate and discard if signs of cracking, drying, or contamination or darkening or hemolysis of the blood are observed. Then label the plate with the information requested by the protocol (typically two patient identifiers and the date). Inoculation procedures vary according to the type of specimen:

i. *Swabs:* Roll the swab specimen across an area approximately one-fifth of the agar (2 x 3 cm) near the edge of the plate. Press the swab firmly on the surface but do not break the agar. Be sure that the entire surface of the swab, including the trip, makes contact with the agar. Use a wire or plastic loop to spread the inoculum over the remainder of the plate, lifting the loop off the plate between the first, second and third streaks (see Figure). Proceed to use the same loop to make stabs perpendicular to and into the agar in the center of the freshly inoculated area of the plate two, then stab two times into the center of the un-inoculated area of the plate.

ii. *Selective or enrichment broth:* Mix the broth thoroughly by vigorously rotating or inverting the broth 10-15 times or by vortexing 10-15 seconds. Transfer a very small droplet (approximately 10 µl) with a wire or plastic loop onto the plate and spread with the same loop over an area approximately one-fifth of the agar (2 x 3 cm) near the edge of the plate. Then use the loop to spread the inoculum over the remainder of the plate, lifting the loop off the plate between the first, second and third streaks (see Figure). Proceed to use the same loop to make stabs perpendicular to and into the agar in the center of the freshly inoculated area of the plate, then stab two times into the center of the un-inoculated area of the plate.

iii. *Filter paper:* Remove the filter paper strip with a sterile forceps (flamed in the burner) and lay it with the inoculated face-down on the surface of the SBA plate, close to the edge of the plate. Moisture from the plate should completely saturate the filter paper; no large air bubbles should remain beneath the filter paper. Incubate the plate aerobically for 4-5 hours at 37°C. Remove the paper with a sterile forceps and lay it down for a few seconds on the middle third of the same plate. Finally, transfer the filter paper onto the remaining third of the same plate, face down, and incubate at 37°C overnight.

iv. *Silica gel transported swabs*: Silica gel transported swabs can be plated directly (see above), but should then be placed in a broth medium and incubated for a minimum of 4 hours; overnight incubation is also satisfactory. If the direct plating of the silica gel swab yields no β-hemolytic streptococci, the swab incubated in broth can be used to inoculate the blood agar plate in the same way as a fresh swab. It is important to express some of the liquid from the swab. If the swab has been incubated overnight, a wire or plastic loop can be used to inoculate the blood agar plate similar to the enrichment broth technique.

v. *Liquid specimens (e.g., pus from abscess)*: inoculate directly into enrichment broth. If the collected sample is small, also rinse out the syringe with 1 ml of the same medium. For detection of bacteria in fluid samples likely to contain low bacteria colony counts, such as body fluids (if >1 mL in volume is provided), the specimen can first be concentrated by centrifugation.

Figure: Differential plating technique for primary cultures of β-haemolytic streptococci

e. Incubation of plates

Plates should be inverted (agar side up) and incubated overnight at 35- 37°C in aerobic conditions. Some investigators believe that identification of GAS may be increased by incubation in an atmosphere containing 5-10% CO_2 or under anaerobic conditions, but these extra steps add cost and are not essential for recovery of GAS. Plates that show no growth of β-hemolytic streptococci after overnight incubation can be incubated for an additional 24-48 hours, but it is generally uncommon for this additional incubation to result in increased recovery of GAS.

f. Recognition of β-hemolytic streptococci on agar plates

i) <u>Presumptive identification</u>

a) *β –hemolysis*: appears as a ring of complete clearing of the blood in the medium surrounding a colony.

b) *Colony morphology:* GAS appears as mostly round β-hemolytic colonies in three main forms: mucoid, matt, and glossy. The colonies are non-pigmented, translucent, usually white to grayish in appearance, and > 0.5 mm in diameter at 24 hours incubation. On certain blood agar media, beta hemolytic streptococci may form larger, white, almost pigmented appearing colonies very similar in appearance to hemolytic staphylococci. This is especially true of group B streptococci. If this occurs, the catalase test can be used to quickly and inexpensively differentiate streptococci from staphylococci; streptococci are catalase-negative and staphylococci are catalase-positive.

ii) <u>Confirmatory tests</u>

Confirm β-hemolytic, gram-positive cocci in pairs and chains as *Streptococcus pyogenes* by *one or both* of the following:

a) *Serogrouping*: Colonies of GAS are often indistinguishable from those of other groups of β-hemolytic streptococci especially groups C and G; serological methods are required for definitive identification. Serogrouping using Lancefield hot acid streptococcal extracts and

immune rabbit sera in a capillary precipitin test is the gold standard method. Other reliable extraction methods have also been used. However, all of these methods relying on a precipitin reaction require a pure culture either in broth or on an agar plate. Thus, identification is delayed by at least one day.

b) *Agglutination test:* Several commercial slide agglutination tests are available to distinguish GAS from other β-hemolytic streptococci, such as groups B, C, F and G. The vast majority of β-hemolytic group A streptococci are *Streptococcus pyogenes*. Occasionally strains of the *Streptococcus anginosus* group (formerly known as *S. milleri*) can be both β-hemolytic and have the group A (or C, F, or G) antigen. However, this is rare and these organisms, normally classified with the viridans streptococci, are not thought to be associated with the serious infections (or sequelae) caused by GAS. These organisms generally grow poorly under aerobic conditions and form small, "pinpoint" colonies that often produce a distinct caramel-like odor. Presence of *Streptococcus anginosus* group organisms carrying group A antigen in the throat flora can result in false positive reactions in rapid antigen detection tests. In culture, the distinctive phenotypic characteristics described above help differentiate them from S. pyogenes. In addition, *S. anginosus* group streptococci have been found to be PYR negative and resistant to 0.04 unit bacitracin differential discs. Therefore, these tests can be used to differentiate *S. anginosu*s group streptococci from *Streptococcus pyogenes*.

g. Preservation of GAS isolates

It may be desirable to store isolates in order for genetic testing or other characterization to be performed in the future. Storage of GAS on blood agar plates when kept at refrigerator temperature in a sealed Petri dish to avoid drying should be limited to less than two weeks. Streptococcal strains can be preserved through freezing or lyophilization (freeze drying).

Quality Management

A quality management plan should be written at the start of the study to assess adherence to the study protocol and standard operating procedures. The plans should include both ongoing "real time" oversight of daily activities and periodic reviews of all study components.

a. Ongoing review

Each clinical and laboratory form should be checked by study personnel other than the one(s) who completed the form. Review should occur as quickly as possible after the forms are filled out to determine whether all required fields are completed, whether appropriate data recording techniques were used (single lines through corrections, legible entries, etc), and whether there are logical inconsistencies in the source data. Both the person completing the form and the person performing the quality check should sign and date the form.

b. Periodic reviews

In addition, a sample of subject charts and microbiology records should be systematically reviewed by a third party on a regular basis (e.g., 2-3 times per year, with the first review close to the onset of the study) to ascertain that all components of the patient record are present and complete. A plan for performance of this quality control should be decided upon prior to the beginning of the surveillance effort. Examples of procedures that could be part of a review are: documentation of informed consent for each participant, if applicable; frequency of forms with omissions or errors in key, predetermined fields; proportion of swabs that are plated within 6 hours of collection; documentation that quality control was performed on each batch of agar plates prepared or purchased; maintenance of logs to monitor temperatures of refrigerators and freezers where media, reagents, and specimens are stored, etc. The results of this audit should be discussed with the team and corrective measures taken if necessary.

References

(1) Carapetis JR, Connors C, Yarmirr D, Krause V, Currie BJ. Success of a scabies control program in an Australian aboriginal community. *Pediatr Infect Dis J.* 1997;16:494-499.
(2) The Working Group on Severe Streptococcal Infections. Defining the group A streptococcal toxic shock syndrome. Rationale and consensus definition. *JAMA.* 1993;269:390-391.
(3) McDonald M, Towers R, Fagan P et al. Recovering streptococci from the throat, a practical alternative to direct plating in remote tropical communities. *J Clin Microbiol.* 2006;44:547-552.

Contributors: Bernie Beall, Alan Bisno, Ann Bouvet, James Campbell, Jonathan Carapetis, Thomas Cherian, Androulla Efstratiou, Richard Facklam, Dwight Johnson, Ed Kaplan, Diana Martin, Malcolm McDonald, Fran Rubin, Stan Shulman

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